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THE TOXIC ACTION OF SCARLATINAL AND PNEUMONIC SERA ON PARAMŒCIA.*

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THE toxic effect of the blood serum of certain animals upon paramœcia was studied by Ledoux-Lebard.† He used the serum of the guinea pig, rabbit, goat, horse, sheep, beef, calf, white rat, pigeon, and goose, diluting with water at least twenty parts to one of serum, in order to render the solution isotonic with the paramœcia. Balbiani had already pointed out the fact that paramœcia would die in any serum which had not been diluted by more than an equal volume of water, this being due, not to substances in the serum toxic to paramœcia, but to its hypertonicity. According to Balbiani, paramœcia are isotonic with a 0.3 per cent. solution of common salt.

Ledoux-Lebard found that the blood serum of all the animals he tested had a more or less pronounced toxic effect upon paramœcia. This effect was shown, first, by a slowing of the movements; then, by an irregular, often rotating movement, which gradually ceased altogether, the paramœcia lying motionless at the bottom of the glass. Careful examination showed that this change in the activity of the paramœcia was due in the beginning

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to the formation of a long viscous thread at the caudal extremity, which impeded progression and caused the paramœcia to make irregular movements in the effort to get rid of it. This filament Ledoux-Lebard regards as a mass of excrement. In most instances the paramœcia became agglutinated by means of their filaments, but this was not invariably true, and was influenced by factors other than the presence of filaments and the slowing of the paramœcia, for it was absent in several cases when these two conditions were present. Ledoux-Lebard thinks that some reciprocal attraction must be present before agglutination can occur. After the paramœcia have become motionless, certain further changes occur, if the serum is toxic enough to cause their death. Their bodies become deformed, the contractile vesicles cease pulsating and dilate, the shape becomes oval, and death ensues. This was seldom earlier than after twenty-four hours. Agglutination occurred usually in two to three hours, slowing, in about fifteen minutes. In case all of the paramœcia did not die, the surviving ones were not found to have acquired immunity. The serum of guinea pig, white rat, goose, and cattle was found toxic in dilutions as high as 1:320; that of white rat and horse produced agglutination in the higher dilutions only. The serum of goose caused immobilization in a dilution of 1:320, more rapid in proportion to the dilution. Beef, rat, and goose serum had the highest degree of toxicity.

He also examined twenty-eight specimens of human serum, fourteen of which were from patients suffering from various diseases, fourteen from the freshly severed umbilical cord of normal parturient women. Six of the first fourteen were non-toxic; eight were toxic in dilutions of 1:20. Of the sera from the parturient women ten were very slightly toxic; four caused immobilization and death of almost all paramœcia within twenty-four hours. This variability was no greater than that seen in the individual cases among the lower animals, nor does Ledoux-Lebard seem to have found much difference between the normal and pathological sera. There are no details given as to the fourteen patients from whom the pathological sera were taken.

Exposure to a tem erature of 55° C. for ten to thirty minutes

destroyed the toxic effect of rabbit and guinea-pig serum. Horse serum and beef serur required 60° C. The effort to restore this toxicity, by the addition of fresh, non-toxic human serum, failed.

These experiments of Ledoux-Lebard, suggesting as they do a new method of study of elements in the blood serum, seemed worthy, not only of confirmation, but of an attempt at further extension, especially into the domain of pathological human serum. Ledoux-Lebard's experiments in this line were not productive of positive results, yet it seemed possible that the examination of a larger number of specimens might show that the variations met with are not only those which are to be expected in different individuals, but that they are dependent upon the different diseased conditions.

The following studies were made upon the serum from cases of scarlet fever and lobar pneumonia chiefly, with a few cases of erysipelas and septicemia, of each of which only four or five specimens could be obtained.

The technic employed was very simple. Cultures of paramœcia in tap water were kept on hand, and the serum was added directly to this water in a dilution of 1:5, the mixture being made in an ordinary hollow-ground slide, which was then placed in a moist chamber. Ledoux-Lebard's dilution of 1:20 was used at first, but was abandoned for the 1:5 dilution, as this produces all the changes in the same order as the weaker, but much more rapidly. If a dilution of 1:20 is used, the first change, slowing and rotary motion on the part of the paramœcia, may occur almost as quickly as in the stronger solution, but very often this effect is transient, only a few paramœcia die, and the rest recover their active motility. This is what was found to occur usually with normal human serum, and often with pathological serum, which could not then be distinguished from the normal. If, however, a large proportion of serum was used, the difference became marked, for the pathological serum caused death within a few hours, while the normal caused only transient slowing, with perhaps the death of a few individuals.

The phenomena which occur in the paramœcia as the result of the toxic action of a serum were found to be the same as those

described by Ledoux-Lebard, with the exception of the agglutination, which was very rarely seen, probably because of the strong solution used. As already stated, Ledoux-Lebard failed to find it in the stronger solutions used by him, probably because immobilization and death occurred too soon. I could find no evidence of "reciprocal attraction." The agglutination, if present, seemed always due to the mechanical effect of the sticky threads attached to the caudal extremities of the paramœcia.

The first change observed as the result of the action of a toxic serum is that the paramœcium swims less rapidly, no longer darts across the field, but makes its way with some difficulty, and turns from side to side, now and then rotating rapidly for a few seconds, apparently in the effort to relieve itself of the clinging mass of excrement which hinders its movements. Even in this stage a dilation of the contractile vesicles may be observed, in cases where the serum is very toxic, but usually this does not appear until the paramœcia have become immobile and fallen to the bottom of the glass. Motionless individuals with some dilation of the vesicles may still recover, and after several hours may be found normal in appearance and activity. In other cases, if the serum is moderately toxic, they may die without further change; but if the serum is very toxic, they become greatly distorted in shape, the contractile vesicles dilate enormously, and coalesce into one large vacuole, which then forces its way to the surface, pushing the protoplasm to one side. Usually the outer covering is not ruptured, but the vacuole occupies the pointed end of the now pear-shaped paramœcium, the granular protoplasm filling the large, rounded end. The quicker the action of the serum, the greater the deformity produced. Paramœcia which die from starvation, from putrefaction in the culture medium, or from heat do not show deformity and seldom show dilation of the vesicles. In this connection may be mentioned an observation of Salomonsen* on the negative chemotaxis exerted by dead paramœcia. This experimenter killed, by means of a hot needle, one or two individuals among a large number of paramœcia, and noticed that

* *Contributions to Celebrate the Inauguration of the State Serum Institute*, Copenhagen, 1902.

the living ones avoided these dead bodies, turning sharply away when they had approached within a certain distance. As I had never observed this avoidance of the dead bodies on the part of the living paramœcia in any of my examinations, I repeated Salomonsen's experiment with the hot needle, and found the result described by him, but found also that it was transient, lasting never more than fifteen minutes. After that the negative chemotaxis, whatever its nature, ceases to exert an influence, and the paramœcia swim indifferently around and over their dead comrades. It would seem that the effect must be produced by the hot needle, for it does not occur when paramœcia die from the effect of toxic substances.

A series of experiments as to the toxicity of the sera of different animals was first made, in confirmation of the experiments of Ledoux-Lebard. The effect in all these experiments is denoted toxic when all paramœcia are killed within twenty-four hours, incompletely toxic when some are still living at the end of this time, or when, although none have been killed, still the transient slowing and dilation have been observed. The degree of toxicity is shown by three asterisks when the full effect takes place within three hours' time, by two asterisks when it takes place in more than three and less than seven hours, and by one asterisk when it occurs between seven and eighteen hours. Incomplete toxicity is expressed by the combination of an asterisk and a line, and absence of toxicity by a line. The following results were obtained:

TABLE I.

The Toxic Action on Paramœcia of Serum of Lower Animals.

Rabbit.....	1	***	Beef	1	***
	3	***		2	***
	4	***		3	*** Serum
	5	***		3	* Blood
	6	***		4	***
	7	***	Sheep	1	**
	8	***		2	*
	9	***	Monkey.....		***
	10	***	Goat.....		*
Dog.....	1	***	Chicken.....		–
	2	*	Hog.....		–
	3	***	Guinea pig...	1	***

It was found that the toxicity of blood was very much less than that of serum, and that serum left in contact with the clot sometimes showed an increase in toxicity after twenty-four hours. As a rule, the toxicity remained unchanged for the first two days, and then diminished progressively, disappearing between the third and fifth days. Heated to 55° C. for thirty minutes, all of these sera lost their toxic effect except the beef serum, in which case the heating had to be prolonged to one hour and a quarter before the toxic action disappeared completely.

The toxic substances are absorbed by the paramœcia, and, as shown by the following experiment, a serum can be rendered non-toxic by the addition of repeated doses of paramœcia:

TABLE II.

Exhaustion of the Toxicity of Serum.

Rabbit serum No. 8 - - - - 1 gtt.
Paramœcium culture - - - 3 gtt.
Death in thirty minutes.
Paramœcium culture - - - - 1 gtt.
Death after eighteen hours.
Paramœcium culture - - - 1 gtt. (making the usual 1:5 dilution).
No effect after twenty-four hours.
This serum was decanted off and fresh serum 1:5 added to the living paramœcia.
Paramœcia died in forty minutes.
The same results were obtained from beef serum.

In order to determine whether the bodies of the dead paramœcia can take up this toxic substance, those killed in the last experiment were exposed to the action of fresh serum for an hour and a half, the serum then decanted and dropped upon living paramœcia. It was shown to have lost none of its toxicity; the bodies of paramœcia killed in this way cannot, therefore, absorb the active substance.

The sera of thirty-four adults in apparently normal condition were then examined (see Table III).

Thus among thirty-four normal sera nine were more or less toxic, three of these nine being markedly so without any apparent cause, as the individuals from whom the blood was taken were in perfect health. Those marked * caused a transient slowing and

swelling of the paramœcia, but no lasting effect. Serum No. 23 was examined four times, and only once exhibited any toxicity. Heating to 55° C. destroyed the toxic action in these nine sera.

One hundred specimens of serum from scarlet-fever patients were then examined, with the results shown in Table IV.

An analysis of these figures shows that of the one hundred sera eighty-five were toxic; the remaining fifteen were either non-toxic or only transiently or incompletely so. In one of these, blood was used instead of serum (203*b*), and, as had been found in the case of the serum of lower animals, the blood was almost inactive. Five of the fifteen non-toxic specimens of serum were

TABLE III.

The Toxic Action on Paramœcia of Normal Human Serum.

No. 1 ...	–	No. 10 ...	–	No. 19 ...	–	No. 27 ...	–
2 ...	**	11 ...	–	20 ...	–	28 ...	**
3 ...	*	12 ...	*	21 ...	–	29 ...	–
4 ...	***	13 ...	*	22 ...	–	30 ...	–
5 ...	–	14 ...	*	23 ...	***	31 ...	*
6 ...	–	15 ...	–	24 ...	**	32 ...	*
7 ...	–	16 ...	–	25 ...	–	33 ...	–
8 ...	–	17 ...	***	26 ...	*	34 ...	*
9 ...	–	18 ...	*				

taken comparatively late in the course of the disease (207, 196, 202, 199, 242), and in all but one of these the sera had proved toxic during the early stages of the disease. Another (206) was from a case in which there was great doubt as to the diagnosis, leaving eight non-toxic cases (204, 205*a*, 190, 220, 221, 262, 278, and 285*a*) which must be regarded as exceptional, as they were taken from cases of undoubted scarlet fever early in the course of the disease. Fifty-eight of the eighty-five toxic cases belonged to the group marked ***; that is, they produced the full effect, within three hours; fifteen were slightly less toxic, taking from three to seven hours to produce their effect; and twelve were still less toxic, taking from seven to eighteen hours. Of these twelve only five were sera taken early in the course of the disease; the remaining seven were either blood, not serum (229 and 230), or were taken late from cases in which the serum had formerly been very toxic (202, 230, 263, 272*b*, and 277*b*). As a rule, the blood

TABLE IV.

Paramœcidal Action of Scarlatinal Serum.

Case No.	Day of Disease	Degree of Toxicity of Serum	Character of Disease	Remarks
178.....	?	* * *	?	Ser. sent in from outside hosp. No hist.
179.....	?	* * *	?	"
181.....	5th	* * *	Severe	
182.....	6th	* *	Moderately severe	
184.....	4th	* * *	Mild	
185.....	5th	* * *	Severe	
186.....	?	* * *	Moderately severe	
188.....	?	* * *	?	"
190.....	4th	–	Severe	
192.....	3d	* * *	Mild	
193.....	13th	* * *	Moderately severe	
194.....	5th	* * *	Moderately severe	
195.....	3d	* * *	Moderately severe	
196*a*	5th	* *	Mild	
196*b*	6th	* *	Mild	
196*c*	12th	–	Mild	
197.....	7th	*	Mild	
198.....	15th	* *	Moderately severe	
199*a*	6th	* *	Moderately severe	
199*b*	25th	–	Moderately severe	
200.....	4th	* *	Moderately severe	
201.....	7th	* * *	Mild	
202*a*	7th	* * *	Moderately severe	
202*b*	11th	* * *	Moderately severe	
202*c*	13th	*	Moderately severe	
202*d*	19th	–	Moderately severe	
203*a*	7th	*	Moderately severe	
203*b*	10th	±	Moderately severe	Blood
204.....	?	–	?	Ser. sent in from outside hosp. No. hist.
205*a*	6th	±	Moderately severe	
205*b*	16th	* * *	Moderately severe	
206.....	6th	±	Mild	Diagnosis doubtful
207*a*	5th	*	Moderately severe	
207*b*	25th	–	Moderately severe	
208*a*	3d	* * *	Moderately severe	
208*b*	10th	* * *	Moderately severe	
209*a*	3d	* * *	Moderately severe	
209*b*	10th	* * *	Moderately severe	
210*a*	5th	* * *	Moderately severe	
210*b*	15th	* * *	Moderately severe	
220.....	?	±	Mild	
221.....	5th	–	Moderately severe	
226.....	6th	*	Moderately severe	
229*a*	3d	*	Mild	Blood
229*b*	3d	* * *	Mild	
230*a*	8th	* * *	Mild	
230*b*	12th	*	Mild	Blood
230*c*	12th	* * *	Mild	
230*d*	13th	*	Mild	
231.....	3d	* *	Moderately severe	
234.....	2d	* *	Moderately severe	
235.....	2d	* *	Moderately severe	

TABLE IV.—*Continued*

Paramœcidal Action of Scarlatinal Serum.

Case No.	Day of Disease	Degree of Toxicity of Serum	Character of Disease	Remarks
236.....	3d	* * *	Mild	
237.....	4th	* * *	Severe	
240.....	4th	* * *	Mild	
242.....	22d	–	Severe	Uremia
245.....	4th	* *	Severe	
249*a*	4th	* * *	Moderately severe	Strep. in blood during life
249*b*	9th	* * *	Moderately severe	
250.....	?	* * *	?	Ser. sent in from outside hosp. No hist.
251.....	4th	* * *	Mild	
252.....	3d	*	?	"
253*a*	5th	* *	Moderately severe	
253*b*	32d	* * *	Moderately severe	
254.....	5th	* * *	Mild	
255.....	7th	* * *	Mild	Diagnosis doubtful
256.....	4th	* * *	Severe	
257.....	?	* * *	?	
258.....	8th	* * *	Severe	
260.....	?	* * *	Moderately severe	
261.....	3d	* * *	Moderately severe	
262.....	3d	±	Mild	
263.....	21st	*	Mild	
267.....		* * *	Mild	
269.....	2d	* * *	Mild	
270*a*	2d	* * *	Severe	
270*b*	4th	* * *	Severe	
271*a*	2d	* * *	Moderately severe	
271*b*	7th	* *	Moderately severe	
272*a*	15th	* * *	Severe	
272*b*	24th	*	Severe	Blood taken 8hr. after death. Strep. in b.
273.....	3d	* * *	Mild	
274.....	3d	* * *	Moderately severe	
275.....	4th	* * *	Severe	
277*a*	4th	* * *	Moderately severe	
277*b*	17th	*	Moderately severe	
278.....	4th	±	Moderately severe	
280.....	3d	* * *	Mild	
283.....	5th	* * *	Very mild	
284*a*	5th	* *	Moderately severe	Comp. with diph.
284*b*	7th	* * *	Moderately severe	
285*a*	3d	* *	Severe	
285*b*	6th	–	Severe	
286*a*	3d	* * *	Mild	Died on day b. was taken. Strep. in blood abundantly.
286*b*	5th	* * *	Mild	
286*c*	8th	* *	Mild	
288.....	3d	* * *	Moderately severe	
289*a*	3d	* * *	Moderately severe	
289*b*	5th	* * *	Moderately severe	
290.....	7th	* * *	Severe	

withdrawn early in the course of the disease was very toxic, and there was a gradual diminution of toxicity, with complete disappearance at varying periods, sometimes as early as the twelfth day (196), although a high degree of toxicity might persist up to the thirty-second day (253), or there might even be an increase, the second sample being more toxic than the first (253, 205).

It is easy to see from the table that the severity of the clinical symptoms had nothing to do with the toxicity of the serum, for a mild case sometimes yielded a serum much more toxic that of a fatal case (compare 273 mild, with 285*b* fatal). Has the streptococcus anything to do with the toxicity of the serum? Paramœcia live and multiply in milk and bouillon cultures of streptococcus, as well as in sterile milk and bouillon; indeed, this organism seems to serve as food for them, for they multiply actively in hay infusion with which an agar culture of streptococcus has been mixed. Balbiani considers the ordinary bacteria of hay infusion the best nutrient for paramœcia, and apparently streptococcus can also be used as food, although putrefactive bacteria cause death of paramœcia in a few hours. Not only could no harmful action be laid to the streptococcus, but it was found that the growth of this organism actually diminished the toxicity of a normally toxic serum. Rabbit's blood serum has been shown to be toxic to paramœcia, and to remain so for twenty-four to forty-eight hours, even when kept at room temperature; but if this serum be inoculated with streptococcus and left for eighteen to twenty-four hours at room temperature, the toxic substances will have disappeared altogether. The same change takes place within the body of the animal. Two rabbits and one guinea pig were given intravenous injections of bouillon cultures of streptococci, isolated from the tonsils of three cases of scarlet fever. After the death of the animals the blood serum was found to be absolutely non-toxic. In the case of the rabbit and the guinea pig, therefore, it would seem that the substance toxic to paramœcia, far from being increased by the growth of the streptococcus, is usually destroyed by it. As for human serum, the presence of streptococci in the blood was demonstrated in cases 249, 272*b*, and 285*b*. The first of these sera was toxic in the highest degree, the second—a fatal

case—only slightly toxic, the third absolutely without effect. In these cases, therefore, it is impossible to attribute any action to the streptococcus. The sera seem to behave exactly as do the sera in which no organisms were found: 249, taken in the early stages was very toxic; 272*b*, in the latter, was less so; 285*b*, non-toxic on the sixth day of the disease, must be reckoned among the unexplained exceptions.

Three cases of septicemia, in two of which the streptococcus was grown from the blood during life, were tested, and also three cases of erysipelas, with the following results:

TABLE V.

	Erysipelas.					Puerperal Septicemia.				
1	-	-	-	-	–1	-	-	-	-	–
2	-	-	-	-	–2	-	-	-	-	±
3	-	-	-	-	±3	-	-	-	-	-

In other words, these six acted exactly like normal human serum, which is usually almost or quite non-toxic; and it is therefore impossible to say, as can be said of the normally paramœcidal sera of the rabbit and guinea pig cited above, that the streptococcal infection had destroyed the toxic substance.

The next question which suggested itself was whether the toxicity of scarlatinal sera would be affected by the streptococcus in the same way as is that of rabbit serum when inoculated with this organism. The sera from two cases of scarlet fever were inoculated with streptococci, and the cultures allowed to grow for twenty-four hours at room temperature.

EXHAUSTING TOXIC SUBSTANCES BY MEANS OF STREPTOCOCCUS.

Serum 195 (twenty-four hours old): paramœcia killed in twenty-four hours.

Serum 195, with twenty-four hours' streptococcus growth: no effect in twenty-four hours.

Serum 230*c* (twenty-four hours old): paramœcia killed in twenty-four hours.

Serum 230*c*, with twenty-four hours' streptococcus growth: no effect in twenty-four hours.

Apparently, then, the growth of the streptococcus in human serum outside of the body has the same effect as the growth in rabbit and guinea-pig serum within and without the body.

Of course it was possible that the effect produced on paramœcia by scarlet-fever serum might also be produced by the serum in other infectious diseases. As the sera from pneumonia patients were available in comparatively large numbers in the laboratory, these were chosen for purposes of comparison. Eighty specimens of serum from pneumonia patients were tested.

The toxicity of pneumonic serum varies greatly, but shows on the whole less divergence from normal serum than does scarlatinal, as can be seen by a comparison of the three:

TABLE VI.

	* * *	* *	*	*	–
Scarlet fever.....	58	15	12	6	7
Pneumonia.....	26	12	8	19	15
Normal.....	3	3	3	6	19

Eighty-five per cent. of the scarlet-fever sera were toxic, 65 per cent. of the pneumonia, and 26.5 per cent. of the normal.

The toxicity of the serum in pneumonia bears no apparent relation to the stage of the disease or to the severity of the symptoms. The serum may be exceedingly toxic as late as the fourteenth day, or it may be non-toxic from the outstart, and this in cases rapidly fatal. The only thing which seemed to have a rather constant influence upon the serum in this respect was the presence of the pneumococcus in the blood. I am indebted for these details to Dr. Rosenow, who was at the time making a bacteriologic study of the blood of pneumonia, and who kindly gave me his results. It was found that only 26 per cent. of the blood sera which had proved toxic to paramœcia gave a fairly abundant growth of the pneumococcus, while 56 per cent. of the non-toxic contained abundant pneumococci.

These results can only be considered as suggesting the possibility that the toxicity for paramœcia of the blood serum in pneumonia is diminished or exhausted by the presence in the blood of large numbers of pneumococci, in the same way apparently as the normal toxicity of rabbit and beef serum and of scarlatinal serum is destroyed by the growth of the streptococcus.

The statement has already been made that the active substance in the blood sera of the lower animals which are toxic to paramœcia is destroyed by heating to 55° C., in some instances to 48°, and that it disappears spontaneously if the serum is left at room temperature for eighteen to twenty-four hours. This was found to be true of scarlet-fever serum also and of pneumonic serum; and further experiments served to show that the toxic substance is not simple, but is composed of at least two bodies, one of which is unstable, easily destroyed by heating to 48° or 55° C. (complement), and disappearing spontaneously after eighteen to thirty-six hours; the other stable, not easily destroyed by heat, and capable of uniting with a fresh complement to produce the toxic effect after the original complement has been destroyed.

Ledoux-Lebard suggested that this was the probable nature of the toxic principle in the blood serum of the lower animals, but he was not able to supply, with fresh non-toxic human serum, the complementary body of the serum of horse, guinea pig, rabbit, etc., which had been subjected to a heat of 55° to 60° C. for thirty minutes. It is possible, however, to reactivate the heated sera of these animals, with fresh serum of another species, and also to reactivate heated scarlatinal serum, although many attempts prove failures, and a fresh, non-toxic serum which serves to supply the complement to one heated serum often fails to do so for another of the same species.

The technic employed in reactivating serum was the following:

TABLE VII.

Paramœcia (*ca.* 200) in water - - - - -	2.0 c.c.
Dog serum heated to 55° C. for thirty minutes	0.4 c.c.

Left over night in ice-box. Paramœcia lively next morning.

Poured into large vessel of tap-water and allowed to swim about for fifteen to thirty minutes. Fished out and placed in hollow-ground slide with normal human serum No. 6 (which had proved to be entirely non-toxic) in proportion of 1 part serum to 5 parts water. Paramœcia were slowed in thirty minutes. Dilation of vesicles and immobilization occurred in one hour and a half, death in two hours.

In the same way beef serum heated to 55° C. for one hour and a quarter was reactivated by three different specimens of fresh non-toxic human serum (Nos. 6, 8, and 11). The above experiment with dog serum heated to 55° and normal human serum No. 5 was repeated, but with this difference, that the human serum was three days old. The result here was negative, the three-day old serum failing to reactivate the heated serum.

The following experiment shows that low temperature temporarily suspends paramœcidal action:

TABLE VIII.

Fresh serum of dog	0.4 c.c.
Paramœcia in water	2.0 c.c.

Left overnight in ice-box. Paramœcia lively next morning, but on removing to room temperature they died in one and one-half hours. Repeated experiment with the same result.

REACTIVATION OF STREPTOCOCCUS SERUM.

a) Beef serum (streptococcus culture, eighteen hours)	1 part
Paramœcium culture	5 parts

No effect in two and one-half hours.

b) Beef serum (streptococcus culture, eighteen hours)	1 part
Paramœcium culture	10 parts
Normal human serum No. 6	1 part

Paramœcia showed slowing and swelling in thirty minutes, death in two and one-half hours.

REACTIVATION OF HEATED SCARLATINAL SERUM.

Serum No. 195 (heated to 55° C. for fifteen minutes)	0.4 c.c.
Paramœcium culture	2.0 c.c.

Left overnight. Paramœcia lively next morning. Poured into a dish of water and left for thirty minutes. Fished out and placed in hollow-ground slide with one part of fresh, normal human serum No. 12 to five parts of paramœcium culture. Death of all paramœcia in fourteen hours.

This experiment was repeated with this difference, that the normal serum was added directly to the mixture of heated scarlatinal serum and paramœcium culture with the addition of enough water to make the dilution the usual 1:5. The result here was the same as in the first experiment, and in the following experiments this simple technic was the one usually employed:

TABLE IX.

Reactivation of Heated Scarlatinal Serum.

Case No. of Scarlatinal Serum	Toxicity	Degree of Heat	Fresh Non-Toxic Serum	Result
201.....	* *	56° C.	10	Dead in eighteen hours
201.....	* *	56	206†	" " " "
202.....	* * *	56	10	" " " "
208.....	* * *	56	202*d*†	Dead in two and one-half hours
209.....	* * *	56	202*d*†	Dead in twenty-four hours
218.....	* * *	56	221†	" " " "
271*a*.....	* * *	48	26	Dead in eighteen hours
272*a*.....	* * *	48	28	" " " "
272*a*.....	* * *	56	28	" " " "
272*a*.....	* * *	60	28	" " " "
288*b*.....	* * *	48	34	" " " "
288*b*.....	* * *	56	34	" " " "
288*b*.....	* * *	60	34	" " " "

† Non-toxic scarlatinal serum.

On the other hand, it was found impossible to restore the toxicity in a large number of cases. For instance, serum No. 99, which had restored the toxicity to No. 209, failed to do the same for Nos. 205 and 210; and there were many similar failures encountered in the effort to find suitable complements for the different sera.

The following experiment shows that the complementary body involved in producing the death of paramœcia is probably the same as that involved in hemolysis of rabbit corpuscles:

TABLE X.

Rabbit corpuscles washed and brought up to original quantity of blood by the addition of normal salt solution - - - - - 2.0 c.c.
Serum - - - - - - - - - - - - - - - - 2.0 c.c.

added at once and then in smaller quantities until hemolysis seemed complete, the tube being kept at 37° C.

This serum, with its hemolytic complement for rabbit corpuscles thus exhausted, was added to paramœcia in the usual 1:5 dilution (allowance being made for the original dilution of the serum with normal salt), and it proved to have no effect on the paramœcia, its toxicity having entirely disappeared, while the control specimen of this same serum, No. 254, was toxic in two and one-half hours.

It has already been stated that the paramœcia which survived the action of a moderately toxic serum had not acquired any immunity thereby, but succumbed to a second dose of the same serum. Efforts were made to immunize the paramœcia against scarlatinal serum by adding repeated small doses of such serum to cultures of paramœcia at intervals of two to three days. The serum used in the first experiment was No. 202, and it was added in doses of 0.5 c.c. to 8.0 c.c. of the paramœcium culture which was kept in the ice-box. Three doses were given, at intervals of two days each. On the third day, after the last dose, the paramœcia were apparently perfectly normal. They were then treated with fresh serum No. 202, in the usual dilution of 1:5, and at the same time a control preparation was made with fresh paramœcium culture and serum No. 202. The paramœcia which had been treated with successive doses died in two and one-quarter hours, the control paramœcia a little later. The same negative results were obtained with serum No. 201. Heated serum was then used, first only three doses being given, then five, then seven; but in all these experiments the paramœcia thus treated showed

themselves, if anything, more susceptible to the usual dose of toxic serum than the untreated paramœcia.

The fact having been demonstrated that there are certain substances in the blood serum in scarlet fever toxic to paramœcia, the question then arose: Are those substances peculiar to the blood of this disease? Is there any qualitative difference between them and the toxic substances present in pneumonia and other diseases? Two lines of experiment were undertaken to answer this question.

In the first place, it seemed possible that there was a difference in the complementary bodies entering into the toxic principle in these two kinds of sera. As has been stated already, the complement in scarlatinal serum which is concerned in the destruction of paramœcia seems to be the same as that which is concerned in the hemolysis of rabbit corpuscles. A parallel experiment was made with pneumonic serum; the hemolytic complement for rabbit corpuscles was exhausted and the serum (formerly toxic to paramœcia) tested. In this case, just as in the case of the scarlatinal serum, the complement had been exhausted, and there was no toxic action on paramœcia, so that this attempt to prove a difference between the two bodies failed.

The second attempt was also a failure. It was thought that by heating the sera to different temperatures it might prove that the serum of scarlet fever could not be reactivated after a certain temperature had been reached, while the serum of pneumonia could still be reactivated, or *vice versa*. Experiments were made with eight scarlatinal and eight pneumonic sera. They were heated to 40°, 48°, 54°, and 60° C. The toxicity in all was destroyed at 48°, and the efforts to reactivate gave no results which pointed to any difference in the two kinds of serum. The reactivation, as always, was capricious; one eighteen-hour-old normal serum would supply the complement for a certain serum and not for others, but there was no apparent difference in the two kinds tested. Both scarlatinal and pneumonic sera can be reactivated after having been heated to 60° for thirty minutes, provided the right normal serum is found.

Mention has already been made of the fact that the blood of the lower animals proved to be less active than the serum. If the serum was removed from the clot immediately after coagulation and left on ice for twenty-four hours, it was found to be less toxic

than the same serum left in contact with the clot. The same was found to be true of the serum of scarlet fever and of pneumonia. The degree of toxicity of the blood never exceeded the lowest—death after seven hours—and in those cases where blood and serum from the same specimen were tested separately, the serum was always much more toxic (Case 229, third day, blood *, serum ***; Case 230, twelfth day, blood *, serum ***). Nor was the full toxic effect obtained if the blood was defibrinated and the serum separated by centrifugal action. Only when the serum was left on ice in contact with the clot for twelve to twenty-four hours was the full effect obtained, and in a few cases an even longer time seemed necessary. Three specimens of serum were most actively paramœcidal at the end of thirty-six hours.

These facts indicate that the leucocytes play an important part in the formation of the paramœcidal substance, and that this substance is probably liberated only after the disintegration of the leucocytes. Certainly such disintegration seems to increase the amount of toxic substance in the serum. The experiments in reactivation of heated sera, described above, tended to show that the element contributed by the leucocytes is the complementary body, for in these experiments it was found that normal human serum when perfectly fresh was not as rich in complement as the same serum kept on ice for twelve to eighteen hours. In some cases the older serum served to reactivate the heated serum when the fresh serum had failed; in others there was simply an increased activity in the older serum.

If, then, the leucocytes supply the complement which enables the toxic substance to attack the paramœcia, might there not be an explanation for the loss of toxicity observed in the sera inoculated with the streptococcus and in the cases of pneumonia which proved to have pneumococci in the blood? May not the streptococcus and the pneumococcus, as they develop, use up the leucocytic substances, and thus deprive the serum of its toxicity by depriving it of this particular elementary body? This theory would seem to be justified by the fact that loss of toxicity caused by the growth of the streptococcus is due to the loss of complement, as shown by the above-described experiments in the reactivation of such sera by fresh non-toxic serum.

CONCLUSIONS.

The serum of many animals (rabbit, guinea pig, dog, beef, sheep, monkey) is toxic to paramœcia.

Normal human serum is usually non-toxic, or very slightly toxic to paramœcia. Nine out of thirty-four specimens were more or less toxic.

The serum of scarlet fever is almost always toxic to paramœcia (85 per cent. of all cases examined).

The serum in pneumonia was toxic to paramœcia in 66 per cent. of all cases examined.

No qualitative differences in the toxic principle in the serum of scarlet fever and in that of pneumonia could be discovered.

The toxicity of scarlatinal serum is apparently independent of the streptococcal infection, being certainly not increased by the presence of the streptococcus in the blood during life, and decidedly diminished or destroyed by the growth of the streptococcus in the blood after death.

The toxicity of the serum in pneumonia is apparently diminished or destroyed by the presence in the blood of the pneumococcus in large numbers.

The paramœcidal substance in all these sera is composed of two different elements; the one being stable, resisting heat of 60° C. for thirty minutes or longer, persisting for at least three days in a serum kept at room temperature, and not affected by the action of the streptococcus within or without the body; the second being unstable, destroyed by heating to 60° C. for thirty minutes or more (in the case of scarlatinal and pneumonic sera to 48°), disappearing spontaneously after eighteen to forty hours at room temperature, and being exhausted or destroyed by the growth of the streptococcus.

The unstable complementary body can sometimes be supplied by the fresh serum of another species, normally non-toxic to paramœcia.

Experiments tend to show that the complement is chiefly or altogether contained in the bodies of the leucocytes, and exists free in the serum only after disintegration of the leucocytes.